BULLETIN

OF THE

Chicago Academy OF Sciences.

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MICRO-CHEMISTRY OF FATS.

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Before the beginning of this century investigations concerning fats and oils were confined almost entirely to the preparation of a few soaps, plasters and burning fluids. The action of alkalies on fats was explained by assuming that the two classes of bodies united without excluding any thing. Even the fact that a metallic compound, as lead acetate, could precipitate an alkaline soap, seemed to suggest little. Scheele, in 1779, separated glycerine from olive oil, and later, from other fats; but, like Priestley, wedded to the old Phlogiston theory, he failed to perceive the great importance of his discovery.

The first satisfactory explanation of the nature of fatty bodies was furnished by the work of Chevreul, commenced in 1811, and by the analyses of others, who, taking up the investigations suggested by this chemist, soon showed the general correctness of his views. Fats were thus proven to be ethers of organic acids, in which glycerine was usually the basic part, and saponification, accordingly, consisted in replacing this glycerine by a metallic oxide. The investigations of Wurtz on the composition of glycerine, and of Berthelot and Heintz on the synthesis of fats, bring this part of the subject down to the present time.

The constitution of these bodies being settled, it now remained to study their various reactions, and improve methods of separation and identification.

We may regard the following formulas as established:

		Molecular weight.
Glycerine	$(C_3 H_5) O_3 H_3$	92
Stearic Acid	$(C_{18} H_{35} O_2) H$	284
Palmitic Acid	$(C_{16} H_{31} O_2) H$	256
Oleic Acid	$(C_{18} H_{33} O_2) H$	282
Butyric Acid	$(C_4 H_7 O_2) H$	88
Caproic Acid	$(C_6 H_{11} O_2) H$	116
Tri-stearin	$C_3 H_5 (C_{18} H_{35} O_2)_3$	890
Tri-palmitin	$C_3 H_5 (C_{16} H_{31} O_2)_3$	806
Tri-olein	$C_3 H_5 (C_{18} H_{33} O_2)_3$	884
Tri-butyrin	$C_3 H_5 (C_4 H_7 O_2)_3$	302
Tri-caproin	$C_3 H_5 (C_6 H_{11} O_2)_3$	386

With very few exceptions, all natural fats consist of mixtures of the last five bodies, and numerous processes have been suggested for separating the constituent parts.

A rough separation can be effected by partial crystallization from some menstruum, or by melting and cooling gradually, in which case the more solid fats separate first.

Fats containing volatile acids can be treated in this way: They are first decomposed by saponification, and treated with dilute sulphuric acid to throw out the free fatty acids. The volatile ones, as butyric, can now be distilled off, or washed out by hot water, in which they are soluble. Methods of analyzing butter are based on these principles. The separation of stearic, palmitic and oleic acids from each other is a problem of very great difficulty, and can be best accomplished in this way, as given by Oudemans (*Fres. Zeit. 1867, p. 454.*) After saponification and precipitation of the fatty acids, an alcoholic solution of the latter is prepared, from which lead acetate throws down the lead salts. These are dried and treated with ether, in which oleate of lead is soluble, while the stearate and palmitate are not. The acids can be separated from the lead readily and weighed, or further treated. The method is not very accurate, as the lead oleate is not easily soluble in cold ether, while, if the latter is warmed, small amounts of the other salts pass into solution.

Palmitic and stearic acids are separated from each other by the process of Heintz. The mixture is dissolved in hot alcohol, and treated with a hot alcoholic solution of magnesium acetate, in such proportion that only about one-seventh of the amount necessary for total precipitation is added. Under these circumstances nearly pure magnesium stearate separates.

From the residue a second and third precipitates are made, and so on. The final ones consist chiefly of magnesium palmitate.

By decomposing all the fractions and repeating the process, it is possible to obtain the one acid in several portions practically free from the other. By uniting and weighing the fractions the desired result is obtained. This process was proposed by Heintz in 1851 (*J. B., 1851, p. 639*), and modified by himself and others later.

As an analytical method it is seldom followed, but for obtaining the pure acids for experimental purposes it answers very well, and was employed by Heintz in preparing the materials for his work on the synthesis of fatty bodies.

The determination of the amount of stearic and palmitic acids in a mixture is usually accomplished by this indirect method.

The molecular weight of stearic acid being 284, and that of palmitic being 256, one C.c. of a $\frac{1}{10}$ normal sodium hydrate solution would neutralize 28.4 Mg. of the former and 25.6 Mg. of the latter. By a simple calculation from the weight of the mixed acids taken, and the C.c. of alkali used, the proportions of the two acids may be deduced. The method is fairly accurate when due precautions are taken. The acids must be dissolved in pure alcohol, or alcohol of known acidity, and a proper indicator, as phenol-phtalein, must be used.

Account must also be taken of the fact that the free acids hold some water, about 3.25 per cent. These equations may be employed in calculating the amounts of acid in such a mixture:

$$\text{Let} \quad x = \text{wt. of stearic acid.}$$
$$y = \text{wt. of palmitic acid.}$$
$$a = \text{wt. of mixture.}$$
$$b = \text{wt. of Na O H used.}$$

$$\text{Then} \quad x + y = a$$
$$\frac{40}{284} x + \frac{40}{256} y = b$$
$$\text{From which} \quad x = 10.143\ a - 64.914\ b$$

A determination of the fusing point of a mixture of the two acids may give some idea of the amounts of each present, but the uncertainties of the method are sufficiently indicated by this table:

Mixture of Stearic.	Palmitic.	Melts.	Solidifies.	Texture.
100	0	69.2	69.2	Crystalline scales.
80	20	65.3	60.3	Fine needles.
60	40	60.3	56.5	Non-crystalline.
40	60	56.3	54.3	Large plates.
30	70	55.1	54.0	Non-crystalline.
20	80	57.5	53.8	Indistinct needles.
0	100	62.0	62.0	Scales.

To be of value these tests must be made with very great accuracy, and both melting and solidifying points must be found.

In practice, the processes mentioned prove of little value in the identification of a majority of the natural fats. In some cases so-called color tests are of use here. These depend on the tints produced by certain reagents when mixed with different fatty bodies, and among the reagents may be mentioned sulphuric acid, nitric acid, zinc chloride, sodium hydrate, hydro-chloric acid and sugar, and several others. At first sight,

one may be pleased by the simplicity of these tests, but on trying a large number uncertainty creeps in, when it is found that various mixtures may be made to yield the colors supposed to be characteristic of some one substance.

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It has been known for a long time that nearly all fatty bodies will crystallize under appropriate conditions, and in forms which are, to a certain degree, characteristic.

The study of a few of these fatty crystals is the main object of this sketch. The literature of the subject is meager and very unsatisfactory. Several authors since Chevreul, Berthelot and Heintz, have referred in a general way to the crystalline forms assumed by natural and artificial fats when crystallizing from various solvents, but evidently, no very great importance was attached to these observations. Attempts were made to distinguish between butter and some of its substitutes by microscopic tests, it being claimed that pure butter was characterized by an absence of small crystals, while other fats, used to adulterate it, possess a multitude of these, especially visible when viewed by polarized light. Some ten years ago, Hassall, in his work on food adulterations, showed the fallacy of this, which fact, however, seems to have been lost sight of by later observers. In a French scientific paper I found, some years ago, reference to the crystallization of certain animal fats, with the statement that from this could be developed a means of identification. Unfortunately, I have lost my memoranda relating to this paper, and can not now recall where I saw it. I think it was published between ten and fifteen years ago, and it was among the first notices relating directly to identification.

In the last five years the subject has received more attention. The following pages embrace the results of my own experiments, commenced in the winter of '82–'83, and which are still in progress. In my investigations I have tried several methods of preparation for microscopic examination.

First Method. Small portions of the fats in question were pressed down to a thin layer between a cover glass and slide, and then examined by ordinary and polarized light, using powers from 75 to 250 diameters. In this way crystals already existing can be detected, but in most cases there is little characteristic about them. Below, under the discussion of butter, it will be seen what use can be made of them.

Second Method. Here a small fragment of the solid fat was melted on a slide, and allowed to cool slowly. In this manner crystals can be obtained, but solidification is usually too rapid to permit large and characteristic forms to appear. By softening the fat by addition of a little olive oil, somewhat more satisfactory results can be obtained, but in

neither case is a free and perfect crystallization possible, as in the following methods.

Third Method. This is the ordinary procedure of crystallization from solution. A small amount of fat was dissolved in ether, carbon bisulphide, chloroform or other menstruum in a beaker or watch-glass, and allowed to stand, until by spontaneous evaporation most of the liquid had passed off. After removing the mother liquor, the deposited crystals were examined on slides in the usual way.

In this way very perfect crystals can be made, but they are not as sharp as when made as follows.

Fourth Method. Here the fats were dissolved as before, and a drop or two transferred to a slide. In a short time, by spontaneous evaporation, a film makes its appearance on the surface of the drop, and at this stage a cover is carefully placed over it, and the slide is allowed to stand until complete crystallization takes place. This requires, in some cases, but an hour, while in others a day or more is necessary. Of all solvents I have found chloroform the most serviceable, and nearly all the crystals described below were made by its use.

I pass now to a discussion of the several fats.

BUTTER.

I have above referred to the opinion once held, that pure butter is composed of non-crystallized fats, and when viewed by polarized light exhibits no refraction phenomena. Hassall and others, as mentioned, have pointed out that on standing, butter soon loses this peculiarity. It has also been found that perfectly fresh butter, and even cream, may sometimes show crystalline forms.

I have been able to confirm these observations as regards butter, but have never found crystals in cream.

When butter is freed from water and salt by melting at a low temperature, and then dissolved in chloroform and crystallized by the fourth method, several forms are observed, the most usual of which are given in Figs. I, and II.*

Crystals like Fig. I, having a diameter of $\frac{1}{250}$ to $\frac{1}{100}$ inch, are very abundant. They consist of fine silky filaments radiating from a center, the field presenting a somewhat wavy appearance, well shown in the figure. Viewed by polarized light the bright crystal on a dark ground appears marked by a cross, as shown under tallow. Fig. II shows another interesting form. We have here a portion resembling Fig. I joined to, or extending into a plate-like bundle of needles similar to some mentioned below.

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* The cuts were made from micro-photographs by the photo-engraving process, with two exceptions. The bees-wax plate and that by polarized light were made from drawings.

In some cases these needles lie so close together as to be undistin-
guishable from plates, with ordinary powers, and are similar to the com-
mon plates of lard or mutton tallow.

Fig. III bears a striking resemblance to Fig. I. The crystals repre-
sented here were obtained from pure stearin, by crystallizing it at a very
low temperature. On allowing a drop of the chloroform solution, on a
slide, to remain over night, the field was found, when examined in the
morning, to be thickly covered with the forms here shown.

Nothing else could be seen, and these disappeared on the application
of a slight degree of warmth. So delicate are they that I made several
attempts to photograph them before succeeding, and then only after
removing my instruments to a cold room. It is possible the butter crys-
tals² may have a similar composition. Other forms observed will be
referred to below.

BUTTERINE.

Samples of butterine, as now found in the market, show a large num-
ber of forms when crystallized from chloroform solution. I have observed
forms very closely resembling Figs. IV, VII, XV and XVI. We have here
a complex body, and the proportions of the several fats used are, without
doubt, of great influence on the manner of crystallization. I wish to
reserve the subject of butterine crystals for a fuller discussion at a future
time, and hence, will not enter into details here.

MUTTON TALLOW.

Mutton tallow and others to follow consist almost entirely of the
glycerides of palmitic, stearic and oleic acids, that is of acids of large
molecular weight, which are not readily volatile without decomposition.
Butter fat differs from these substances in this, that it contains 8 to 10 per
cent. of glycerides of volatile acids. There is necessarily a marked physi-
cal difference between butter and other fats, and we should expect corres-
ponding differences to appear in the microscopic forms. Figs. IV, V, VI,
VII, VIII and IX give the chief forms found in mutton fat. All of these
crystals can be found on one slide, and frequently two or three in the same
field. It is not easy to say which is the most characteristic or numerous.
Nos. IV, V and VI are readily produced, and are, in a certain sense, pri-
mary forms. They are among the first which become clearly defined on
the evaporation of the chloroform, and are very stable, remaining many
months, perhaps years, in perfect shape. Nos. VII, VIII and IX are
peculiar. Crystals separate from chloroform solutions of mutton fat very
quickly, some forming within a few minutes, while others separate out
after a longer time. Very soon a field is found which consists of well
defined rhomboidal plates, Fig. VII, which differ from Fig. IV in this,

that they are shorter, and, as will be seen, less stable. If one watches them carefully, he will find them undergoing a change by splitting in the direction of their greatest lengths into bundles of needles. Soon these diverge from each other at the ends, giving the appearances well shown in Figs. VIII and IX. The divergence is often greater than here shown.

I have followed these changes very closely with the microscope, and in one instance made photographs of two fields to show this.

Fig. VIII is, in fact, an enlarged portion of the crystals of Fig. VII, and was taken four hours later. This change of rhomboidal plates like Fig. VII, to bunches of divergent needles, as shown in VIII and IX, is somewhat characteristic of mutton tallow. Growth and change of crystals can be observed in nearly all fats, but this peculiar change I have not noticed elsewhere.

BEEF TALLOW.

Very fine crystals can be obtained from this substance, but the variety is probably not as great as in the case of mutton fat. The more usual forms are represented in Figs. X, XI, XII and XIII. Very frequently I have observed crystals which differ from X only in this, that the needles terminate without crossing at the center. Figs. XI, XII and XIII are well represented. The last named is one of the commoner forms found in the fat of the dog. In Fig. XI the nucleus is not quite in the center, but I have found in tallow many crystals in which it is in the center, and when such are viewed by polarized light we obtain the effect well given in Fig. XIV, the cross being exactly similar to that referred to under butter. I have occasionally found in pure tallow masses of crystals similar to those of Fig. IV. These are more frequently found when the tallow is mixed with a small amount of fatty oil.

LARD.

Lard differs, qualitatively, very little from beef or mutton tallow, but quantitatively, it is characterized by a larger amount of olein. The microscopic forms are not numerous. Figs. XV and XVI show those most commonly seen. The rhomboidal plates of XV are usually sharply defined and transparent, as they are very thin. They resemble closely the plates found in mutton tallow. In Fig. XVI we have a combination of an imperfect plate with a plumose tuft, somewhat similar to a form found in butter, but not quite the same. Another form seems to consist of very small plates which have undergone a partial change by sending out spines as in some of the mutton tallow crystals. The spines are, however, very short and sharp. It is possible that Fig. XVI grew in a somewhat similar manner. A change, in some degree analogous to this, may be observed by crystallizing beeswax from chloroform. The plates which first form are equilateral rhomboids, but after a time these change in a very remark-

able manner. The points farthest apart become blunt, and send out little spicules. These grow by curving outward until the dumb-bell of Fig. XVII is reached. The first and intermediate forms are also shown in XVII, and the process of change can be readily followed by the micro-scope.

In mixtures of lard and tallow numerous plates resembling XV can be found, and, in general, everything found in each, provided proper propor-tions be taken. When equal parts of the two fats are mixed the crystals formed are frequently of the types shown in Figs. XVIII and XIX. Fig. XVIII is very characteristic, consisting of plates expanded at one end into tufts of curved needles. In Fig. XIX we have a number of over-lying plates, the ends of which have grown into tufts. By varying the propor-tions, very different results can be obtained. When the tallow is largely in excess a very common form is produced, shown in Fig. XX. This form polarizes as XIV. Needles form much more readily than plates.

HUMAN FAT.

In examining the fats above mentioned I took for solution portions representing the average composition of the whole substance. Melted human fat readily separates on cooling, into two layers, a liquid, and a semi-solid mass. For my tests I worked with the latter, which had the consistency of lard.

Two forms are readily found on microscopic examination, and beyond these not much more. These are well shown in Figs. XXI and XXII, of which those like XXI predominate. Sometimes the very fine needles radiate from the center in all directions, and without curvature.

DOG FAT.

The fat of the dog crystallizes from chloroform solution in fine wavy needles, radiating from a center. Fig. XXIII shows a very common appearance. Other forms are similar to fine crystals of butter shown in Fig. I, and to the tallow-lard mixture of Fig. XX. The cross by polarized light is perfectly exhibited.

CAT FAT.

Fig. XXIV shows a form of crystal readily obtained from solutions of cat fat. Other forms consist of imperfectly shaped plates observed in mutton and butter.

It will be observed that butter fat and the last three described have this in common. Their ordinary mode of crystallization is in exceedingly fine lines or needles radiating from a nucleus. The lines of lard, beef-tallow, or mutton-tallow, are not as fine or feathery as those just men-tioned. Butter and the other fats resemble each other, even when very

fresh, in having a strong and characteristic odor, showing the presence of relatively large amounts of volatile substances. These volatile matters are doubtless glycerides of lighter acids, and the addition of a small amount of such substance makes a change in the appearance of the crystals, as I have found by adding a little artificially prepared glycerine butyrate to some mutton tallow.

OTHER FATS.

In his classic study of the composition of fats Berthelot prepared a number of them artificially, by heating mixtures of glycerine and the fatty acids. (*J. B.*, *1853*, *p. 451*.)

By heating equal weights of glycerine and stearic acid in a sealed glass tube to a temperature of 200°C. for 26 hours, he obtained, after eliminating the uncombined acid and glycerine, mono-stearin. In like manner he prepared di-stearin and tri-stearin, and the glycerides of other acids.

Soon after this Heintz went through the same processes, obtaining like results. (*J. B.*, *1854*, *p. 447*). According to the process proposed by Berthelot, I have prepared several of the fats, and studied their crystalline forms. The results have not been altogether satisfactory, or just what I anticipated. There are many difficulties in the way of purifying some of the products when prepared, but until this is done I think we cannot expect to reach a fair explanation of the many forms observed in these crystallizing bodies.

Several hypotheses may be presented to account for the numerous modes of crystalline aggregation observed in the photographs I have made, but it must be admitted that no one of them amounts to a satisfactory explanation. It is usually held that the ordinary phenomena of di- and tri-morphism, observed among mineral crystals, are brought about by variations of temperature at time of crystallization. It is also known that several simple substances of perfectly definite composition crystallize in different forms from different solvents.

Among the simpler fatty bodies I have noticed a remarkable variety of forms in samples of pure stearic acid, prepared by fractional precipitation, and in pure stearin.

If we assume that these samples were not absolutely pure, which is of course probable, there comes up another hypothesis worthy of consideration. Williamson, in advancing his theory of etherification (*Chem. Soc. Quar. Jour.*, *IV*, *229*), and Clausius in discussing certain phenomena of electrolysis (*Pogg.*, *CI*, *338*), proposed an explanation of the nature of solutions, which has been of great assistance in the study of many reactions. They held that the component parts of a molecule in solution can not be considered as firmly linked to each other, but as continually moving about, exchanging places with corresponding parts of other molecules.

KCl and $NaNO_3$ in solution form some $NaCl$ and KNO_3, so that at any one time we may consider four substances present, instead of two. This hypothesis may be extended to molecules of greater complexity.

Referring to the formulas on page (74), we see that we have in most natural fats three groups:

$$C_3 H_5 \begin{cases} C_{18} H_{35} O_2 \\ C_{18} H_{35} O_2 \\ C_{18} H_{35} O_2 \end{cases} \qquad C_3 H_5 \begin{cases} C_{16} H_{31} O_2 \\ C_{16} H_{31} O_2 \\ C_{16} H_{31} O_2 \end{cases} \qquad C_3 H_5 \begin{cases} C_{18} H_{33} O_2 \\ C_{18} H_{33} O_2 \\ C_{18} H_{33} O_2 \end{cases}$$

Tri-stearin.   Tri-palmitin.   Tri-olein.
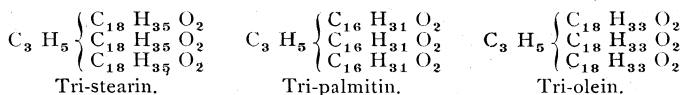
In solutions, more could be formed from these, as:

$$C_3 H_5 \begin{cases} C_{18} H_{35} O_2 \\ C_{18} H_{35} O_2 \\ C_{16} H_{31} O_2 \end{cases} \qquad C_3 H_5 \begin{cases} C_{18} H_{35} O_2 \\ C_{18} H_{35} O_2 \\ C_{18} H_{33} O_2 \end{cases} \qquad C_3 H_5 \begin{cases} C_{18} H_{35} O_2 \\ C_{16} H_{31} O_2 \\ C_{18} H_{33} O_2 \end{cases}$$
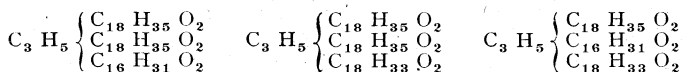
If we consider a fourth or a fifth fat present, as in the case of butter, a much greater number of combinations would be possible, and I think it probable that at the time of crystallization some of these secondary forms would separate out, just what ones, depending, of course, on conditions of temperature and concentration. At one instant the conditions may be right for the formation of one nucleus, and at the next for the formation of another.

While many combinations are possible, a few are more probable because of their stability; just as in the cases of isomers among alcohols and hydrocarbons.

I believe that by working at lower temperatures, I could have obtained an even greater variety of crystalline aggregates than indicated above, as was indeed suggested by my experience with stearin. These considerations lead me to think that the microscopic identification of mixtures of fats is a problem of much greater difficulty than might appear at first sight. I think ultimately a great deal may be accomplished in this direction, but until the various effects of temperature and percentage composition are understood; until they have been demonstrated by actual experiment, I think it unwise to lay very great stress on results obtained in this way.
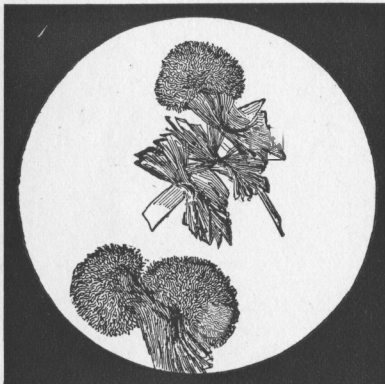
CHICAGO MEDICAL COLLEGE, January, 1885.
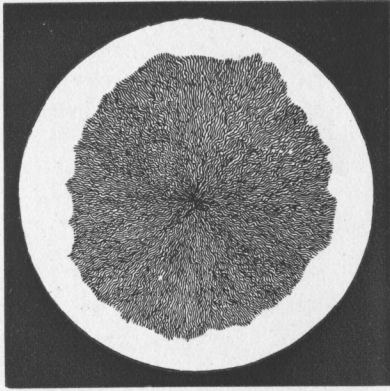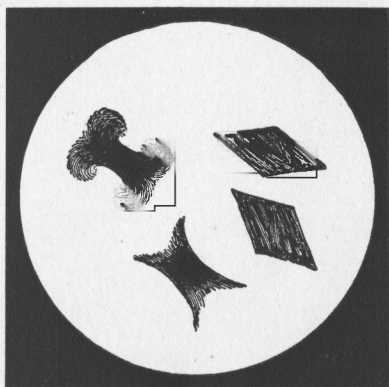
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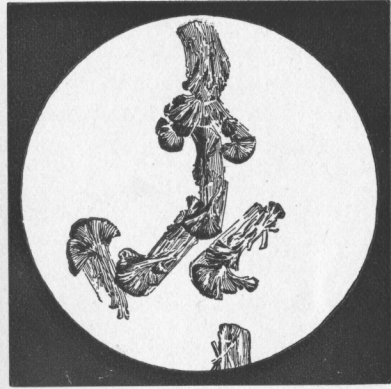
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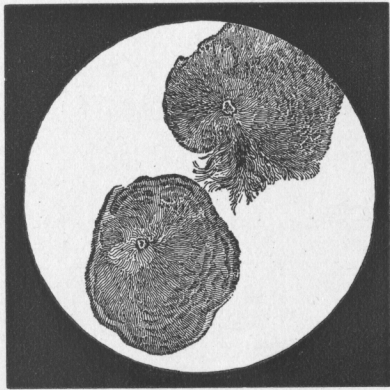
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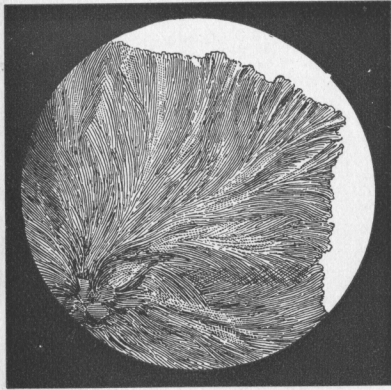
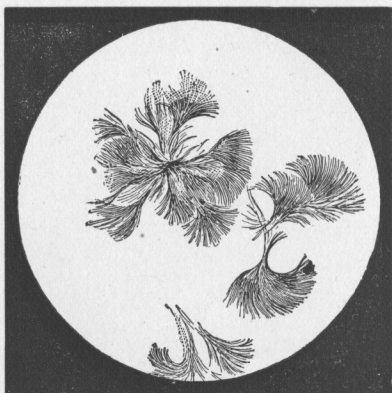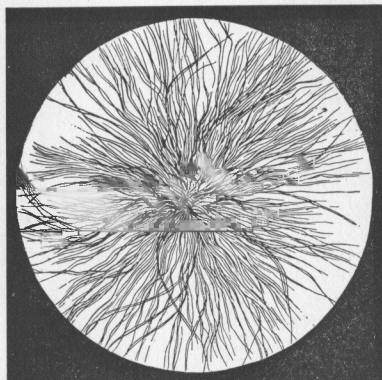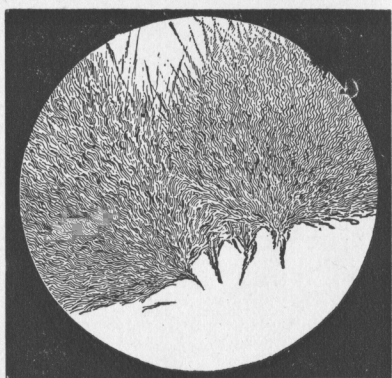IV.
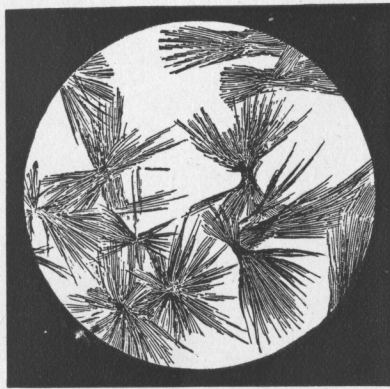
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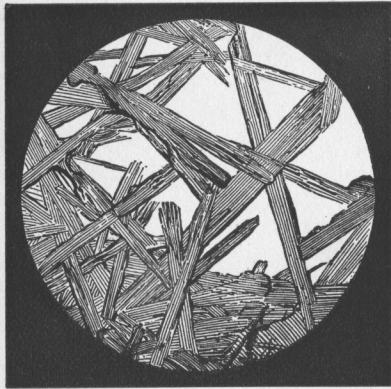
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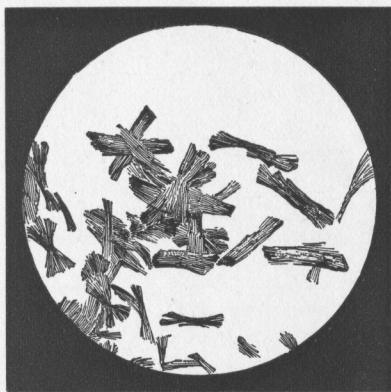
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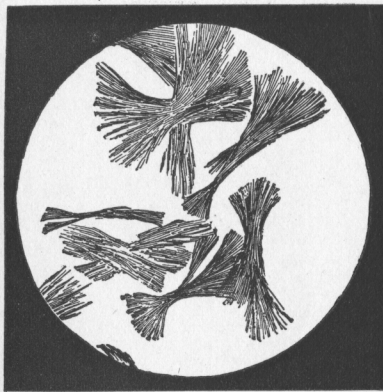
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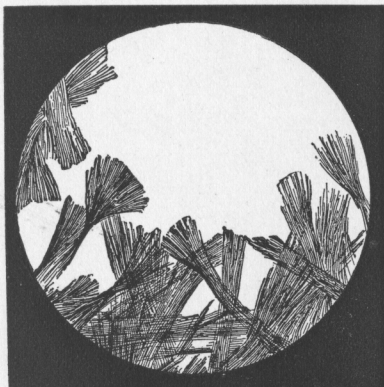
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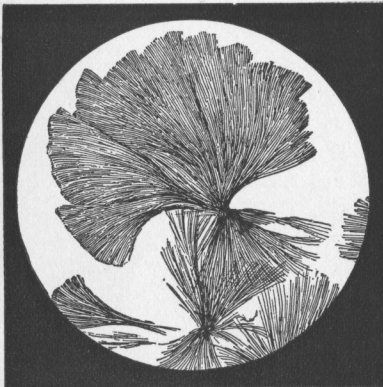
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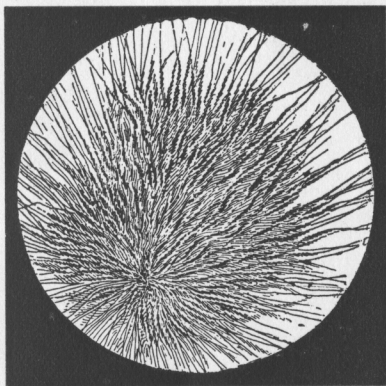
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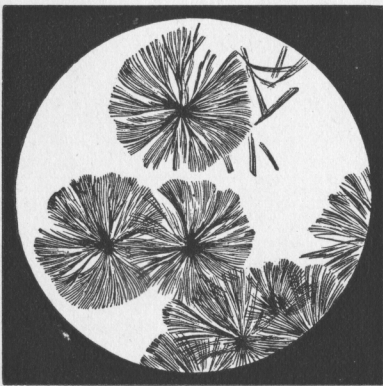
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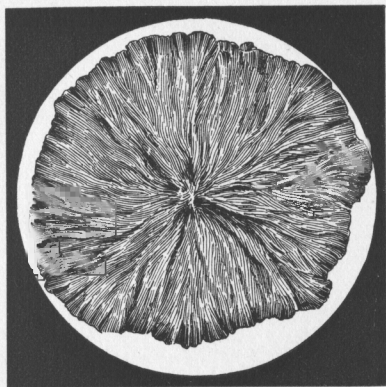
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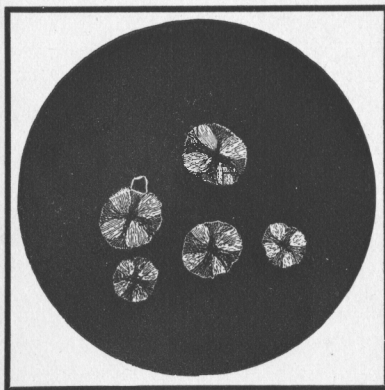
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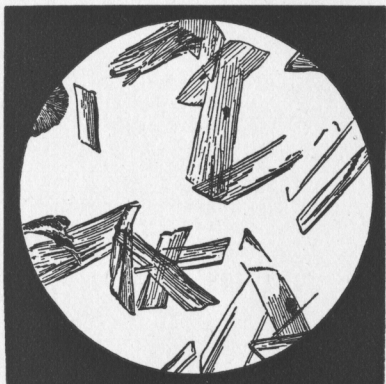
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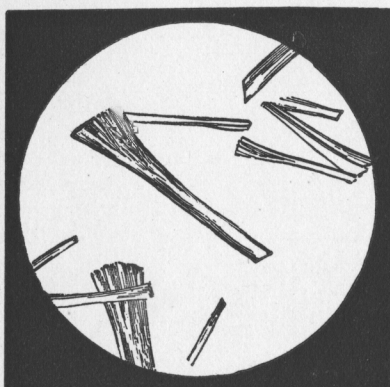
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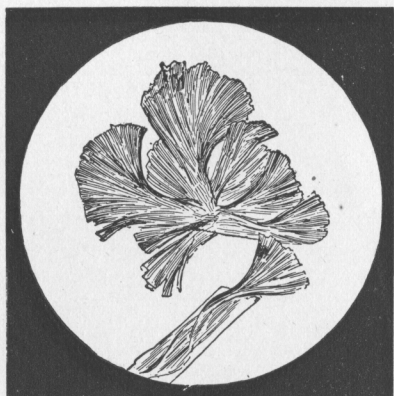
XIX.

XX.

XXI.

XXII.

XXIII.

XXIV.